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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(54) Title: COMPOSITION FOR TREATING ACNE

## (57) Abstract

A method and composition for preventing and treating acne is disclosed. The composition contains an anti-bacterial agent that inhibits the growth of the cutaneous bacteria that are believed to cause acne. In particular, the anti-bacterial agent comprises a bacteriocin produced by *Propionibacterium*. For instance, in one embodiment, the bacteriocin is produced by the B1264 strain of *Propionibacterium jensenii*. The bacteriocins of the present invention can be contained in a topical preparation that is applied to the skin.

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## COMPOSITION FOR TREATING ACNE

5       The present application is based on a  
Provisional Application filed September 13, 1996  
and having U.S. Serial No. 60/026,036.

Field of the Invention

10      The present invention is generally directed to  
a composition and method for treating acne. More  
particularly, the present invention is directed to  
the use of a protein produced by bacteria from  
dairy or classical propionibacteria that inhibits  
the growth of bacteria that cause acne.

Background of the Invention

15      Acne (formally known as "acne vulgaris") is a  
skin disease which afflicts, in some form,  
approximately 80 to 90 percent of all teenagers.  
Acne can also affect many adults and infants. The  
severity of acne can range from minor skin lesions  
20      called comedones to nodules and cysts which can  
potentially permanently scar skin tissue.

25      The areas of the body that are commonly  
affected by acne are the face, chest and upper  
back. These areas of the body have a high  
sebaceous gland concentration which causes the skin  
to be oily.

30      Acne is believed to start with increased  
release of hormones in the body such as androgens.  
Young adults going through puberty, for instance,  
tend to produce more hormones such as androgens.  
It is believed that androgens stimulate the  
sebaceous glands to produce more sebum, which is an  
oily substance released on the skin. Sebum is a  
lipid-laden product comprised of a mixture of fats,  
35      waxes and fatty materials.

As more and more is produced, sebum can  
agglomerate and form solid plugs known as comedones  
in the hair follicles. Formation of a comedone, in  
turn, causes layers of dead skin known as keratin

to accumulate and be retained within the lining of the follicles. The buildup of keratin causes hyperkeratosis of the follicular opening, which can completely close off the follicular canal.

Within the follicular canal are bacteria indigenous to the follicular lining. Included in the bacteria are anaerobic, gram positive organisms classified for instance as Propionibacterium acnes or Propionibacterium granulosum. When comedones form in the follicular opening, these bacteria feed on the sebum. Specifically, the bacteria produce lipases that hydrolyze triglycerides and release free fatty acids. The fatty acids thus generated in combination with the bacteria activate the immune system of the body causing inflammation, swelling and redness around the follicle. Depending upon the degree of inflammation, pustules, cysts, nodules, and papules may form.

In the past, many different treatments have been proposed for curing acne. Such treatments include topical keratolytic agents, topical or systematic antibiotics, or hormonal treatments designed to limit sebum secretion. Unfortunately, some of these treatments can produce adverse side affects.

Topical keratolytic agents include, for instance, tretinoin and benzoyl peroxide. These anti-acne agents prevent the blockage of follicular canals and reopen clogged follicular openings by breaking down keratin. Some keratolytic agents, however, can irritate the skin causing excessive dryness, scaling, swelling, burning, peeling, redness and allergic reactions.

Antibiotics that have been used in the past to treat acne include tetracycline, erythromycin, clindamycin, mecloxyclin, metronidazole, minocycline and doxycycline. While administration

of these drugs is often effective in treating acne, antibiotics have several disadvantages. For example, when taken orally, antibiotics may cause many undesirable side effects including abdominal cramps, black tongue, fatigue, depression, nausea, and other various effects. When applied topically, the antibiotics can lose their effectiveness if used for a prolonged period of time due to resistance developed by the bacteria.

The hormones estrogen and antiandrogens have also been used to treat acne in severe cases. These hormones when taken orally, however, can feminize adolescent males and can increase the risk of blood clots and cancers. Thus, hormones are rarely administered orally.

In view of the disadvantages associated with current acne treatments, a need exists for an acne preventive agent that does not create any of the above adverse side effects. A need also exists for an anti-acne agent that destroys the bacteria that causes the inflammation associated with acne.

#### Summary of the Invention

The present invention recognizes and addresses the foregoing disadvantages, and others of prior art constructions and methods.

In summary, the present invention is generally directed to an anti-bacterial protein referred to as a bacteriocin, that can be used to destroy and inhibit the growth of the bacteria that are responsible for the inflammatory symptoms associated with acne. According to the present invention, the bacteriocin is produced by a bacteria from the genus *Propionibacterium* and particularly from the classical or dairy strains. For instance, in one embodiment, the bacteriocins can be produced from *Propionibacterium jensenii*, and more particularly by the B1264 strain of

5       Propionibacterium jensenii. It has been discovered  
that these bacteriocins not only can be used to  
treat acne but are stable across a pH range and at  
relatively high temperatures. This stability  
coupled with the fact that the bacteriocin is  
produced by a food related organism, makes the  
bacteriocin of the present invention a highly  
effective and desirous agent for treating acne.

10      Bacteriocins produced by Propionibacterium  
jensenii are discussed in United States Patent No.  
5,639,659 which is incorporated herein by reference  
in its entirety. In the prior application, the  
bacteriocins were used primarily to inhibit the  
growth of bacteria cultures of Lactobacillus  
15     bulgaricus and Streptococcus thermophilus in order  
to control the pH of a food product, such as  
yogurt. It was not disclosed nor was it apparent  
in that application, however, that the bacteriocins  
may also be used to inhibit the bacteria associated  
20     with acne and other medical ailments.

Accordingly, it is an object of the present  
invention to provide a method and a composition for  
treating acne.

25      Another object of the present invention is to  
provide a method and composition for treating acne  
that involves the use of bacteriocins produced from  
one or more strains of bacteria classified in the  
genus Propionibacterium.

30      Another object of the present invention is to  
provide a composition containing bacteriocins  
produced by Propionibacterium jensenii that destroy  
and inhibit the growth of bacteria associated with  
acne.

35      It is another object of the present invention  
to provide a topical preparation for treating acne  
that contains bacteriocins produced by  
Propionibacterium jensenii.

Still another object of the present invention is to provide a method for destroying and inhibiting the growth of Propionibacterium acnes and Propionibacterium granulosum using a bacteriocin produced by Propionibacterium jensenii.

These and other objects of the present invention are achieved by providing a process for treating acne. The process includes the step of 10 treating an infected area of the body with an effective amount of bacteriocins produced by a species of Propionibacterium, such as Propionibacterium jensenii, to destroy cutaneous bacteria responsible for causing acne and other 15 diseases. Specifically, the bacteriocins are capable of inhibiting the growth of acne bacteria such as Propionibacterium acnes and Propionibacterium granulosum. In one embodiment, bacteriocins produced by the B1264 strain of 20 Propionibacterium jensenii are used.

The bacteriocins of the present invention may be used alone to treat acne or in conjunction with other anti-acne agents. In particular, the bacteriocins are believed to be well suited for use 25 with keratolytic agents such as benzoyl peroxide, retinoic acid and salicylic acid.

When applied to the skin, the bacteriocins are preferably combined in a topical composition. As such, the present invention is also directed to an 30 anti-acne composition. Besides containing bacteriocins produced by a species of Propionibacterium, such as Propionibacterium jensenii, the composition can also contain other 35 anti-microbial agents, wetting agents, keratolytic agents, chelators, linolenic acid, preservatives, and drying agents. The composition can contain all or a mixture of some of the above ingredients as

desired.

Other objects, features and aspects of the present invention are discussed in greater detail below.

5

Detailed Description of Preferred Embodiments

It is to be understood by one of ordinary skill in the art that the present discussion is a description of exemplary embodiments only, and is not intended as limiting the broader aspects of the 10 present invention, which broader aspects are embodied in the exemplary construction.

The present invention is generally directed to a composition and method for treating acne and other medical conditions. In particular, a 15 bacteriocin produced by dairy propionibacteria, such as Propionibacterium jensenii, is used to destroy and inhibit the growth of bacteria that cause acne and other medical ailments. Specifically, it has been discovered that the 20 bacteriocins of the present invention inhibit the growth of Propionibacterium acnes and Propionibacterium granulosum, which are cutaneous bacteria present in the follicular canal of the skin.

25

In order to treat acne, the bacteriocins produced by dairy propionibacteria are applied topically in the infected area. As will be described in more detail hereinafter, the bacteriocins can be combined with various 30 ingredients to form a topical composition. Once applied to the skin, the composition destroys the bacteria associated with acne reducing inflammation and infection.

35

Besides acne, the bacteriocins of the present invention, because they inhibit the growth of cutaneous bacteria, can also be used to treat other

medical ailments and diseases. Specifically, the bacteriocins can be used to treat any medical condition caused by cutaneous bacteria, such as the ones identified above. For instance, it is  
5 believed that the bacteriocins of the present invention can also be used to treat endocarditis, brain abscesses, dental infections, conjunctivitis, pulmonary infections, and peritonitis.

A bacteriocin is an anti-bacterial agent comprising a protein or a complex of proteins. The bacteriocins for use in the present invention are derived from bacteria cultures of propionibacteria, such as the classical or dairy species of Propionibacterium. The classical or dairy species include Propionibacterium jensenii, P. acidiropionici, P. freudenreichii subspecies freudenreichii and shermanii, and P. thoenii. These classical species are extremely useful organisms in the food industry and are found in  
10 dairy fermentations, other natural fermentations such as silage and olives, and soil. During fermentative metabolism, they convert glucose and lactate to propionate, acetate, and carbon dioxide.  
15 The inhibitory effects of propionate and acetate are potentiated by the low pH encountered in Swiss cheese and other fermented products. Of particular advantage, the classical or dairy species of propionibacteria are food related bacteria that can be safely consumed in human food.

In one embodiment, bacteriocins produced by the B1264 strain of Propionibacterium jensenii have been found particularly well adapted for use in the process of the present invention. Besides being effective in inhibiting the growth of cutaneous bacteria cultures, bacteriocins produced by the  
20 B1264 strain of Propionibacterium jensenii are very stable. Through testing, it has been discovered

that these bacteriocins can withstand temperatures of up to 100°C for at least sixty minutes, remain active in a pH range from about 2 to about 10, are stable to solutions of sodium chloride, are stable 5 in alcohols, are stable in up to 4M urea solutions and are stable in solutions of anionic detergent sodium dodecyl sulfate. Bacteriocins produced by the B1264 strain of Propionibacterium jensenii have a molecular size of between about 3,000 to about 10,000 Dalton.

Although it is believed that other strains of Propionibacterium, such as Propionibacterium jensenii, may produce bacteriocins that can be used in the present invention, it has been discovered 15 that bacteriocins produced by the B1264 strain are particularly effective at inhibiting the growth of and destroying bacteria cultures believed to be responsible for many of the symptoms associated with acne and other diseases or conditions. These 20 bacteriocins, for instance, have been found to be effective in destroying bacteria cultures of Propionibacterium acnes and Propionibacterium granulosum. More particularly, the bacteriocins have been found to inhibit the growth of 25 Propionibacterium acnes ATCC 6919, Propionibacterium acnes ATCC 11827, and Propionibacterium granulosum ATCC 25564. It has also been observed that the bacteriocins inhibit 30 Propionibacterium avidum ATCC 25577 and Propionibacterium lymphophilum ATCC 27520, which are strains of bacteria commonly present on the skin but not at the present time known to be associated with acne.

It should be understood that the scope of the 35 present invention is not to be limited to the use of bacteriocins produced by B1264 strain of Propionibacterium jensenii. The use of

bacteriocins produced by the B1264 strain of Propionibacterium jensenii merely represents one embodiment of the present invention. It should be understood that the present invention is directed 5 to the use of bacteriocins produced from any strain of Propionibacterium, and particularly to the classical or dairy strains. In a further alternative embodiment, it has also been discovered that bacteriocins produced by Lactococcus lactis 10 subsp. lactis also inhibit cutaneous bacteria and thus can be used in the process of the present invention. As will be demonstrated in the examples to follow, provided herein is a simple screening test for determining whether a particular strain of 15 propionibacteria produces a bacteriocin that will inhibit the cutaneous bacteria responsible for causing acne.

In order to produce the bacteriocins, a particular strain of Propionibacterium is first 20 grown in a suitable medium. As the bacteria grows, the bacteriocin of the present invention is produced. Once produced, the bacteriocin can be isolated and combined in a topical composition for application to the skin. It is believed that only 25 a few molecules of the bacteriocin will be required to kill a cell of the targeted bacteria.

In forming a topical preparation, the bacteriocins of the present invention can be combined with a wide variety of ingredients that 30 can be added in order to facilitate application to the skin, to provide a benefit to the skin, or for some other benefit. For instance, the bacteriocins may be combined with other anti-acne agents, wetting agents, chelators, preservatives, or drying 35 agents. For example, the bacteriocins of the present invention may be used in combination with all types of anti-acne agents that have anti-

microbial properties and/or keratolytic properties.

In particular, the bacteriocins may be used in combination with antibiotics. Of particular importance, however, it is believed that the bacteriocins would be well suited for use with compositions that will break down keratin such as salicylic acid, retinoic acid, and benzoyl peroxide. Benzoyl peroxide is also known to have some anti-microbial effect.

Wetting agents that may be used with the bacteriocins include quaternary ammonium compounds, sodium dodecyl sulfate, polyphosphates, carbopol, propylene glycol, polyethylene glycol, polyoxyethylenesorbitans, and mixtures thereof.

Chelators can be included in the composition for binding with metal ions in order to facilitate the destruction of bacteria cells. Exemplary chelators that may be used in the present invention include EDTA, citric acid, and phosphoric acid. Linolenic acid may be also added to the composition in order to maintain the formulation of sebum in balance on the skin.

The composition of the present invention containing the bacteriocins may also contain preservatives, such as benzoic acid, butylated hydroxy anisole (BHA) and parabens. If desired, drying agents may also be added to the composition, such as alcohols.

The present invention may be better understood with reference to the following examples.

#### EXAMPLE I

The following is one procedure that may be used in order to produce bacteriocins from *Propionibacterium*, such as *Propionibacterium jensenii*, and particularly from the B1264 strain of *Propionibacterium jensenii*.

*Propionibacterium jensenii* B1264 obtained from

the All Union Collection of Microorganisms at the Russian Academy of Sciences in Moscow (48 h, 0.1% inoculum) was added to sodium lactate broth (NLB) and held for 10 days at 32°C under flowing CO<sub>2</sub> (0.4 L/min) to permit culture growth and bacteriocin production. The cells were removed from the cultures by centrifuging at 6,300 × g for 30 min at 4°C and filtering through a 0.45 µm pore-size filter.

The bacteriocins produced were precipitated by adding to the filtered supernates ammonium sulfate (70% final concentration) gradually with constant stirring. The mixture was then held overnight at 4°C. The bacteriocins were agglomerated and formed into pellets by centrifuging at 19,600 × g (30 min).

The pellets containing the bacteriocins were resuspended in 0.1 M sodium phosphate buffer (pH 6.4) at 5% original volume. Preparations to be examined for activity against *P. acnes* may be resuspended at 0.2% of original volume. The solution was dialyzed exhaustively against the same buffer at 4°C using 3,500 molecular weight cut-off dialysis tubing. The dialysis removed salts and other low molecular weight components contained in the solution. The dialyzed preparation was then sterilized by passing it through a 0.45 µm pore-size filter resulting in a more concentrated crude bacteriocin preparation.

30

#### EXAMPLE II

The bacteriocins produced in Example I were then assayed against a reference culture of *Lactobacillus delbrueckii* subspecies *lactis*.

35       *L. delbrueckii* subsp. *lactis* ATCC 4797 was grown overnight in *Lactobacilli* MRS broth at 37°C. Sufficient amounts of the culture were added to 5 mL soft MRS agar (*Lactobacilli* MRS broth containing

0.75% agar) to yield a final population of  $\approx 10^6$  cells per mL. The culture-soft MRS agar mixture was poured over the surface of sterile MRS agar (1.5% agar) plates.

5 Bacteriocins produced by Propionibacterium jensenii according to the procedure of Example I were diluted in serial two-fold dilutions. Dilutions were applied (in 10  $\mu$ L portions) to the surface of the prepared plates. The plates were  
10 held at 37°C under flowing CO<sub>2</sub> (0.4 L/h) for 16-18 hours and examined for zones of inhibition. It was observed that the bacteriocins inhibited the cultures of L. delbrueckii subsp. lactis. Titors were expressed as the reciprocal of the highest  
15 dilution inhibiting the indicator culture and are reported in activity units per milliliters.

Using the procedures described in Examples I and II, any strain of Propionibacterium, such as Propionibacterium jensenii, can be screened and  
20 tested for bacteriocin production. Once bacteriocins are found to exist, they can be easily tested against cutaneous propionibacteria as explained in the following Examples.

#### EXAMPLE III

25 The following represents one method for detecting bacteriocin inhibition against cutaneous propionibacteria contained in cultures. The method is referred to as the deferred agar spot assay modified appropriately for cutaneous  
30 propionibacteria and is particularly well adapted for use with bacteriocins produced by Propionibacterium jensenii.

Each pure culture of possible bacteriocin-producing Propionibacterium (Propionibacterium jensenii) is grown at 32°C for 48 h in sodium lactate broth (NLB). Each producer culture (10  $\mu$ L) is then spot-inoculated to the center of sodium

lactate agar (NLA) plates and held at 32°C under flowing CO<sub>2</sub> for 8 days to permit bacteriocin production.

5           Propionibacterium acnes, Propionibacterium granulosum or other cutaneous propionibacteria to be examined for sensitivity to the bacteriocin are grown in actinomyces broth (Difco Laboratories, Detroit, MI) at 37°C for 2 days under anaerobic conditions (BBL GasPak system). Plates containing  
10 possible bacteriocin-producing cultures are overlaid with 5 mL of soft actinomyces agar (actinomyces broth containing 0.75% agar) containing 15 µL of the culture being examined for sensitivity. To eliminate inhibition by H<sub>2</sub>O<sub>2</sub> or  
15 organic acids, separate filter paper discs containing 15 µL of catalase and pronase E (20 mg/mL) are applied near the growth of the producer culture.

Plates are incubated anaerobically at 37°C for  
20 24-48 hours to permit growth of the culture being examined for sensitivity and are examined for zones of inhibition. Inhibition that is not affected by catalase and is sensitive to protease is considered to be due to a possible bacteriocin.

25           From the above procedure, it was discovered that bacteriocins produced by the B1264 strain of Propionibacterium jensenii inhibited the growth of bacteria cultures of Propionibacterium acnes ATCC 6919, Propionibacterium acnes ATCC 11827 and  
30 Propionibacterium granulosum ATCC 25564.

The above-described procedure may be used to screen any bacteriocin produced by Propionibacterium jensenii against any strain of cutaneous propionibacteria.

35           **EXAMPLE IV**

The following procedure is a method for detecting bacteriocin activity in solution against

cutaneous propionibacteria. The method can be used for screening any bacteriocin produced by Propionibacterium. The following procedures include both the spot on lawn method and the critical dilution method.

5           Propionibacterium acnes, Propionibacterium granulosum, or other cutaneous propionibacteria to be examined for sensitivity to the bacteriocin are grown in actinomyces broth at 37°C for 2 days under anaerobic conditions. Cultures (15 µL) being examined for sensitivity are added to soft actinomyces agar (5 ml) and the mixture is applied uniformly over each actinomyces agar plate.

10           Preparations concentrated so as to contain 6,400 Activity Units/ml of bacteriocins are spotted (25 µl spots) onto the surface of the prepared plates.

15           These plates are held at 37°C for 2 days under anaerobic conditions to permit growth of the cutaneous culture and examined for zones of inhibition (lack of growth) where the bacteriocin was applied. Zones are evidence of bacteriocin inhibition.

20           This method can be modified to permit quantitation of bacteriocin activity against cutaneous cultures by preparing serial two-fold dilutions of bacteriocin solution and applying 25 µL spots from each dilution to the surface of the plates (containing cutaneous propionibacteria cultures in soft actinomyces agar). These plates are held at 37°C for 2 days under anaerobic conditions to permit growth of the cutaneous culture and examined for zones of inhibition (lack of growth) where the bacteriocin was applied.

25           Titters can be expressed as the reciprocal of the highest dilution inhibiting the indicator Propionibacterium culture and are reported in activity units (AU) per mL.

The above-procedure can be used to screen the effectiveness of any bacteriocins produced by *Propionibacterium* against any cutaneous propionibacteria.

**EXAMPLE V**

Bacteriocins produced by the B1264 strain of Propionibacterium jensenii were tested against cutaneous propionibacteria obtained from patients. Specifically, clinical specimens of open comedones or blackheads were obtained from fifteen patients. The comedones were homogenized and cultured on Marshall and Kelsey Medium as described in an article entitled: "Microbiology of Comedones in Acne Vulgaris" by Marples, et al., J. Invest. Dermatol., Volume 60, Pages 80-83 (1973) and New Propionibacteria Isolation Medium as described in an article entitled: "New Medium for Isolating Propionibacteria and its Application to Assay of Normal Flora of Human Facial Skin" by Kishishita, et al., Appl. Environ. Microbiol., Volume 40, Pages 1100-1105 (1980). 150 clinical cultures were isolated and identified by biochemical profiles and fatty acid analysis.

Bacteriocins produced by the B1264 strain of  
25 Propionibacterium jensenii were tested against the  
clinical isolates. The bacteriocins inhibited all  
150 clinical isolates. Of the 150 isolates, 129  
were identified as Propionibacterium acnes and the  
remaining 21 were identified as Propionibacterium  
30 granulosum.

**EXAMPLE VI**

Bacteriocins produced by the P126 strain of Propionibacterium jensenii were also tested against various cutaneous propionibacteria. It was observed that these bacteriocins did not inhibit the bacteria cultures although further testing may be needed to confirm these results. It is

believed, however, that other strains of Propionibacterium jensenii will produce bacteriocins that may be used in the process of the present invention.

5

#### EXAMPLE VII

The following procedure was developed according to the present invention in order to further purify bacteriocins produced by Propionibacterium, especially bacteriocins produced 10 by Propionibacterium jensenii.

A strong anion exchange resin, BIORAD MACRO-PREP Q (typically 6 milliliters packed wet volume), was prepared by washing 5 times with 0.025 M or 0.1 M sodium phosphate buffer (pH 6.0) containing 0.1% 15 TWEEN 80, centrifuging at 6,300 x g for 10 minutes, and decanting supernates. Crude bacteriocins produced by the B1264 strain of Propionibacterium jensenii as prepared in Example I (1 ml, 1600 AU/ml) were dialyzed for 24 h at 4oC in 3500 MWCO 20 tubing against 0.025 M sodium phosphate buffer (pH 6.0). The dialyzed bacteriocins were mixed with the washed resin, and held with gentle stirring for 10 minutes. Contaminating proteins, but not the bacteriocins, adsorbed to the resin. IEC-purified 25 bacteriocins were recovered by decanting the supernate, repeatedly (5 to 6 times) washing the support with 1 milliliter of 0.025 M or 0.1 M sodium phosphate buffer (pH 6.0) containing 0.1% TWEEN 80 and combining the supernate and washings.

30

The above batch anion exchange chromatography procedure was found to yield 145% recovery of bacteriocin activity and a 22-fold increase in purification. Because of the increase in activity, it is believed that the above purification 35 procedure may have removed some agents that were interfering with bacteriocin activity.

EXAMPLES VIII

The following three examples were performed in order to determine the stability of the bacteriocins produced by the B1264 strain of Propionibacterium jensenii when contained in various solvents. In one particular application of the present invention, the bacteriocins may be combined with an alcohol in producing a topical preparation. Thus, the following tests were performed in order to ensure that the bacteriocins would remain active in such preparations. Crude bacteriocins produced by the B1264 strain of Propionibacterium jensenii as prepared in Example I were combined with methanol, ethanol and isopropanol and sterile deionized, distilled water to a final concentration of 0%, 15%, 30% and 50% (v/v). Each mixture was held at 18°C to 22°C for 0, 30, 60, 90 and 120 minutes and examined for activity against L. delbrueckii subsp. lactis ATCC 4797. Under the assay conditions, sodium phosphate buffer (0.1 M, pH 6.0) containing the same solvent concentrations did not inhibit the sensitive indicator culture. Therefore, mixtures were assayed directly for bacteriocin activity.

The following results were obtained:

Table 1  
Effect of Solvents on Crude Bacteriocins

	Solvent	Activity (AU/ml)				
		0 min.	30 min.	60 min.	90 min.	120 min.
5	Methanol 0%	1600	1600	1600	1600	1600
	Methanol 15%	1600	1600	1600	1600	1600
	Methanol 30%	3200	1600	1600	1600	1600
	Methanol 50%	1600	1600	1600	1600	1600
10	Ethanol 0%	3200	3200	3200	3200	3200
	Ethanol 15%	3200	3200	3200	3200	3200
	Ethanol 30%	3200	1600	3200	3200	3200
	Ethanol 50%	3200	3200	3200	3200	3200
15	Isoprop 0%	3200	3200	1600	1600	1600
	Isoprop 15%	3200	3200	1600	1600	1600
	Isoprop 30%	3200	1600	1600	1600	1600
	Isoprop 50%	3200	3200	1600	1600	1600

Ethanol and isopropanol may possibly be used in a topical preparation containing the bacteriocins of the present invention for treating acne. As shown above, neither solvent had a dramatic effect upon the activity of the bacteriocins.

#### EXAMPLE IX

The same procedure described in Example VIII above was also carried out on bacteriocins produced by the B1264 strain of Propionibacterium jensenii that were partially purified by ion exchange chromatography as described in Example VII above.

The following results were obtained:

Table 2  
Effect of Solvents on IEC Purified Bacteriocins

Solvent		Activity (AU/ml)				
		0 min.	30 min.	60 min.	90 min.	120 min.
5	Methanol 0%	400	200	200	200	400
	Methanol 15%	200	200	200	200	200
	Methanol 30%	400	200	200	200	400
	Methanol 50%	200	200	200	200	200
	Ethanol 0%	400	400	200	200	400
10	Ethanol 15%	400	200	200	200	400
	Ethanol 30%	400	200	200	200	200
	Ethanol 50%	400	200	200	200	200
	Isoprop 0%	400	200	200	200	400
15	Isoprop 15%	200	200	200	200	200
	Isoprop 30%	100	100	100	100	100
	Isoprop 50%	100	100	100	100	100

As shown above, none of the solvents had a dramatically adverse impact upon bacteriocin activity, further suggesting that the bacteriocins are well suited for use in topical preparations.

#### EXAMPLE X

The same procedure described in the Example VIII above was repeated except for the fact that the bacteriocins, instead of being examined for activity against *L. delbrueckii* subsp. *lactis*, were examined for activity against *Propionibacterium acnes* ATCC 6919. The following results were obtained:

Table 3  
Effect of Solvents on Bacteriocins  
Assayed Against P. Acne

	Solvent	Activity (AU/ml)				
		0 min.	30 min.	60 min.	90 min.	120 min.
5	Methanol 0%	400	400	400	400	200
	Methanol 15%	400	200	200	400	400
	Methanol 30%	400	400	400	400	400
	Methanol 50%	400	200	200	400	400
10	Ethanol 0%	400	400	400	400	400
	Ethanol 15%	400	400	200	200	400
	Ethanol 30%	400	200	200	200	400
	Ethanol 50%	400	200	200	200	200
15	Isoprop 0%	400	400	400	200	200
	Isoprop 15%	400	400	400	200	200
	Isoprop 30%	400	400	200	200	200
	Isoprop 50%	100	100	100	100	100

As shown above, again, none of the solvents had a dramatically adverse impact upon bacteriocin activity, even when examined for activities against Propionibacterium acnes.

#### EXAMPLE XI

Besides bacteriocins produced by Propionibacterium jensenii, it was also discovered that bacteriocins produced by Lactococcus lactis subsp. lactis also inhibited cutaneous bacteria. These bacteriocins are commercially known as nisin or NISAPLIN.

Specifically, a solution of nisin containing 4,000 International Units per milliliter was tested for activity against Propionibacterium acnes ATCC 6919, Propionibacterium acnes ATCC 11827, Propionibacterium avidum ATCC 25577 and Propionibacterium granulosum ATCC 25564 according

to the method described in Example IV above. It was observed that nisin inhibited all of the cutaneous cultures.

Nisin at a final concentration of 400 International Units per milliliter was also added to broth cultures of each of the above bacteria strains. The broth cultures were incubated anaerobically at 37°C for 24 hours. It was observed that nisin markedly reduced the growth of each culture of cutaneous propionibacteria.

In view of these results, it is believed that nisin may also be used in the process of the present invention as an anti-acne agent or as an agent for treating other diseases caused by cutaneous bacteria.

It should be understood that the present invention is not limited to the specific bacteria or processes described herein and that any bacteriocin equivalent to those described falls within the scope of the present invention. Preparation routes of the bacteriocins and process steps of inhibiting bacteria are merely exemplary so as to enable one of ordinary skill in the art to produce the bacteriocin and use it according to the described process and its equivalents.

It will also be understood that although the form of the invention shown and described herein constitutes a preferred embodiment of the invention, it is not intended to illustrate all possible forms of the invention. In addition, it should be understood that aspects of the various embodiments may be interchanged both in whole or in part. The words used are the words of description rather than of limitation. Various changes and variations may be made to the present invention without departing from the spirit and scope of the invention.

WHAT IS CLAIMED IS:

1. A method for treating and preventing acne comprising the step of:

applying to skin a composition containing an anti-bacterial agent, said anti-bacterial agent comprising a bacteriocin produced by dairy propionibacteria, said bacteriocin inhibiting the growth of cutaneous bacteria that cause acne.

5 2. A method as defined in claim 1, wherein said bacteriocin are produced by Propionibacterium jensenii.

3. A method as defined in claim 1, wherein said bacteriocin are produced by the B1264 strain of Propionibacterium jensenii.

4. A method as defined in claim 1, wherein said composition contains other anti-bacterial agents besides said bacteriocin.

5 5. A method as defined in claim 4, wherein said other anti-bacterial agents comprise a material selected from the group consisting of salicylic acid, retinoic acid, benzoyl peroxide and mixtures thereof.

6. A method as defined in claim 1, wherein said composition further comprises a wetting agent.

7. A method as defined in claim 1, wherein said composition further comprises a chelator.

8. A method as defined in claim 1, wherein said composition further comprises a drying agent.

9. A method as defined in claim 1, wherein said composition further comprises linolenic acid.

10. A topical composition for treating and preventing acne, said composition comprising:

5 at least a first anti-acne agent and a second anti-acne agent, said first anti-acne agent comprising a bacteriocin produced by dairy propionibacteria, said bacteriocin inhibiting the

growth of cutaneous bacteria that cause acne, said second anti-acne agent comprising a material selected from the group consisting of salicylic acid, retinoic acid, and benzoyl peroxide.

11. A composition as defined in claim 10,  
wherein said bacteriocin is produced by  
Propionibacterium jensenii.

12. A composition as defined in claim 10,  
wherein said bacteriocin is produced by the B1264  
strain of Propionibacterium jensenii.

13. A composition as defined in claim 10,  
further comprising a wetting agent.

14. A composition as defined in claim 10,  
further comprising a chelator.

15. A composition as defined in claim 10,  
further comprising a drying agent.

16. A composition as defined in claim 10,  
further comprising linolenic acid.

17. A method for treating and preventing acne comprising the step of:

applying to skin a composition containing an anti-bacterial agent, said anti-bacterial agent comprising a bacteriocin produced by Propionibacterium jensenii.

18. A method as defined in claim 17, wherein said bacteriocin is produced by the B1264 strain of Propionibacterium jensenii.

19. A method as defined in claim 17, wherein said composition further comprises an anti-acne agent, said anti-acne agent being capable of breaking down keratin.

20. A method as defined in claim 17, wherein said composition further comprises a solvent, said solvent comprising an alcohol.

21. A process for controlling the growth of bacteria cultures containing bacteria selected from the group consisting of Propionibacterium acnes and

5           Propionibacterium granulosum, said process  
comprising the step of adding to said bacteria  
cultures a bacteriocin produced by dairy  
propionibacteria.

22. A process as defined in claim 21, wherein  
said bacteriocin are produced by Propionibacterium  
jensenii.

23. A process as defined in claim 21, wherein  
said bacteriocin are produced by the B1264 strain  
of Propionibacterium jensenii.

## INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/16267

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K7/48 A61K35/74 C12N1/38

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 A61K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	STN, file supplier, Karlsruhe, DE, file XP002049833 Biosis, AN=97:195712 see the abstract --- STN, file supplier, Karlsruhe, DE, file XP002049834 Biosis, AN=97:284403 see the abstract ---	1-3,17, 18,21-23
X,P	US 5 639 659 A (BAREFOOT ET AL.) 17 June 1997 cited in the application see the whole document ---	1-3,17, 18
P,A	-/-	1-23

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

## \* Special categories of cited documents :

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \*Z\* document member of the same patent family

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Date of the actual completion of the international search
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11 December 1997

Date of mailing of the international search report
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- 4. 02. 98

Name and mailing address of the ISA
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## INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/16267

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	STN, file supplier, Karlsruhe, DE, file XP002049835 Chemical Abstracts, vol 122, AN=153453 see the abstract ---	1-23
A	STN, file supplier, Karlsruhe, DE, file XP002049836 Chemical Abstracts, vol 116, AN=102317 see the abstract ---	1-23
A	WO 89 12399 A (PUBLIC HEALTH RESEARCH INSTITUTE OF THE CITY OF NEW YORK) 28 December 1989 see the whole document -----	1-23

# INTERNATIONAL SEARCH REPORT

Intern. application No.  
PCT/US 97/16267

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:  
see FURTHER INFORMATION sheet PCT/ISA/210
2.  Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3.  Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1.  As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4.  No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
- No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Remark : Although claim(s) 1-9,17-20  
are directed to a diagnostic method  
practised on the human/animal body , the search has been carried out and  
based on the alleged effects of the compound/composition.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Internati Application No

PCT/US 97/16267

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5639659 A	17-06-97	NONE	
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WO 8912399 A	28-12-89	AT 142504 T AU 631803 B AU 3843089 A DE 68913189 D DE 68913189 T DE 68927189 D DE 68927189 T DK 45690 A EP 0382814 A EP 0545911 A FI 98880 B IE 63998 B IL 90700 A JP 8009525 B JP 3500051 T NO 179354 B US 5304540 A US 5334582 A US 5691301 A US 5135910 A US 5217950 A US 5260271 A	15-09-96 10-12-92 12-01-90 24-03-94 19-05-94 17-10-96 30-01-97 21-02-90 22-08-90 09-06-93 30-05-97 28-06-95 24-06-94 31-01-96 10-01-91 17-06-96 19-04-94 02-08-94 25-11-97 04-08-92 08-06-93 09-11-93
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